Canine Breeding Management

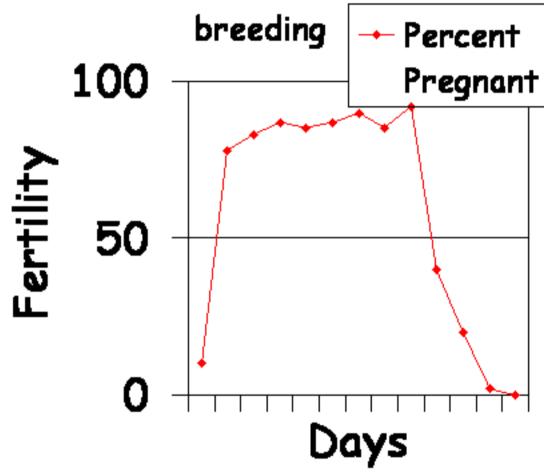




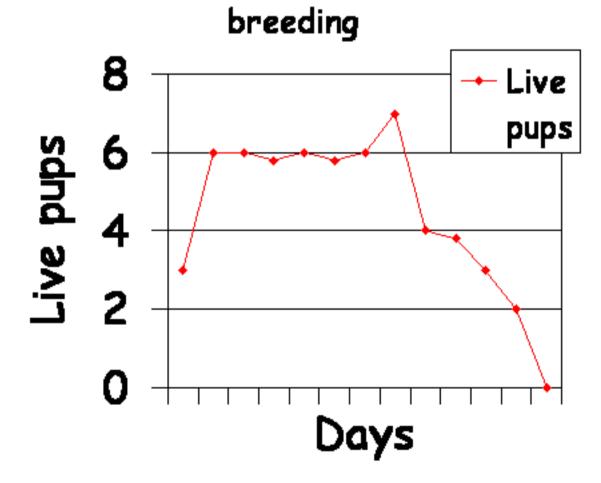
p 41

- Improper timing of breeding is often the cause of unsuccessful breeding.
- The conception rate after a single breeding is highest if the bitch is bred 3-10 days prior to Day 1 of diestrus (D1).
- After a single breeding, the number of live pups per CL is highest when the breeding occurs 4 days prior to D1 by a proven, fertile male. This would equ 9 day estrus.





Fecundity Relative to a single



Fertile Male

- The breeding male should be **Brucella canis** negative.
- The ejaculate should contain at least 200×10^6 total cells
- It should have 80 % motility
- 80% of the the cells should have normal morphology.

Breeding

- If natural breeding is performed, be active in the breeding process. Observe the breeding to see if a tie occurred.
- Do not just leave the dogs together and hope they breed.
- The timing of the breeding is important. If the male and female are both available for unlimited breeding, then breedings can be done every other day the





On the left breeding dogs, on the right the male has turned and 'stepped over' so the dogs are rear to rear. This creates a 90 degree bend in the

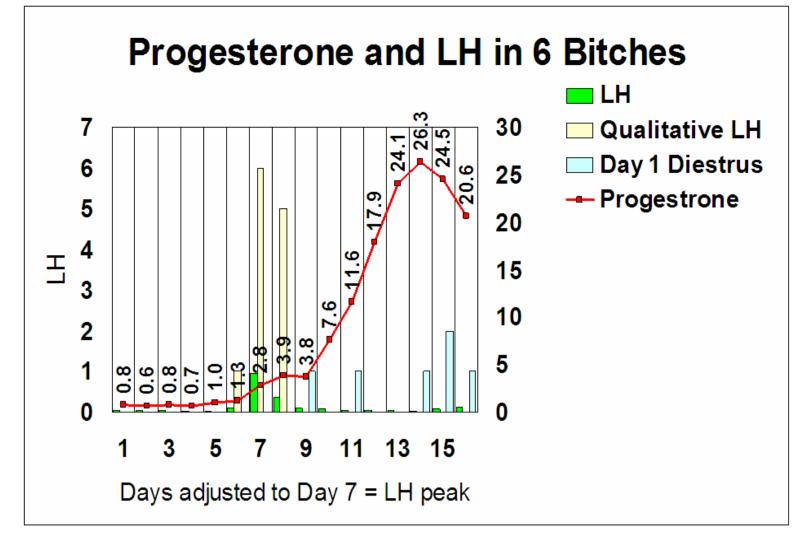
See some more ties by clicking here.

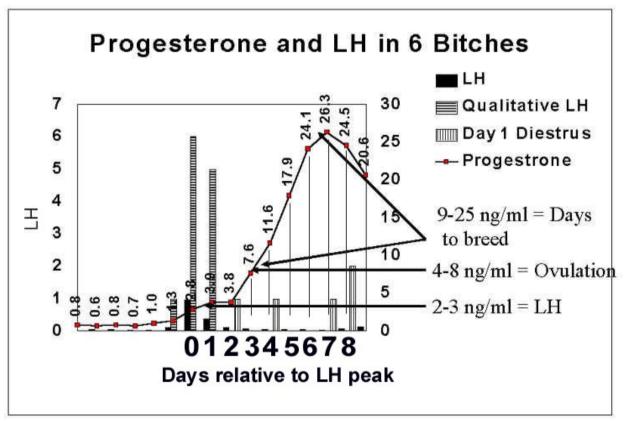
Timing the cycle for optimal breeding





- The estrous cycle should be monitored to establish when the bitch is actually in estrus.
 - This is important because there are several reasons why breeding may not occur, or the fertile period may be missed.
 - Some reasons include: male female incompatibility caused by physical problems in either the male or female.
 - Psychological problems.
 - Behavioral problems.
- If there are constraints on the availability of the male, or the number of breedings possible, it is important that the cycle be monitored carefully to ensure
- Management of breedings using shipped, chilled semen or frozen semen require excellent timing of the fertile period.
- Methods for timing a breeding include:
 - Behavior of the bitch, such as flagging the tail and standing to be mounted.
 - Physical signs such as:
 - Vulvar swelling
 - Bloody discharge.
 - Vaginal cytology
 - Ultrasound examination of ovarian follicular activity
 - Hormone assay for either progesterone or LH.
 - While vaginal cytology is fairly reliable, all other methods are unreliable except for hormone assay.
- Hormone assays are the best way to determine the fertile period.





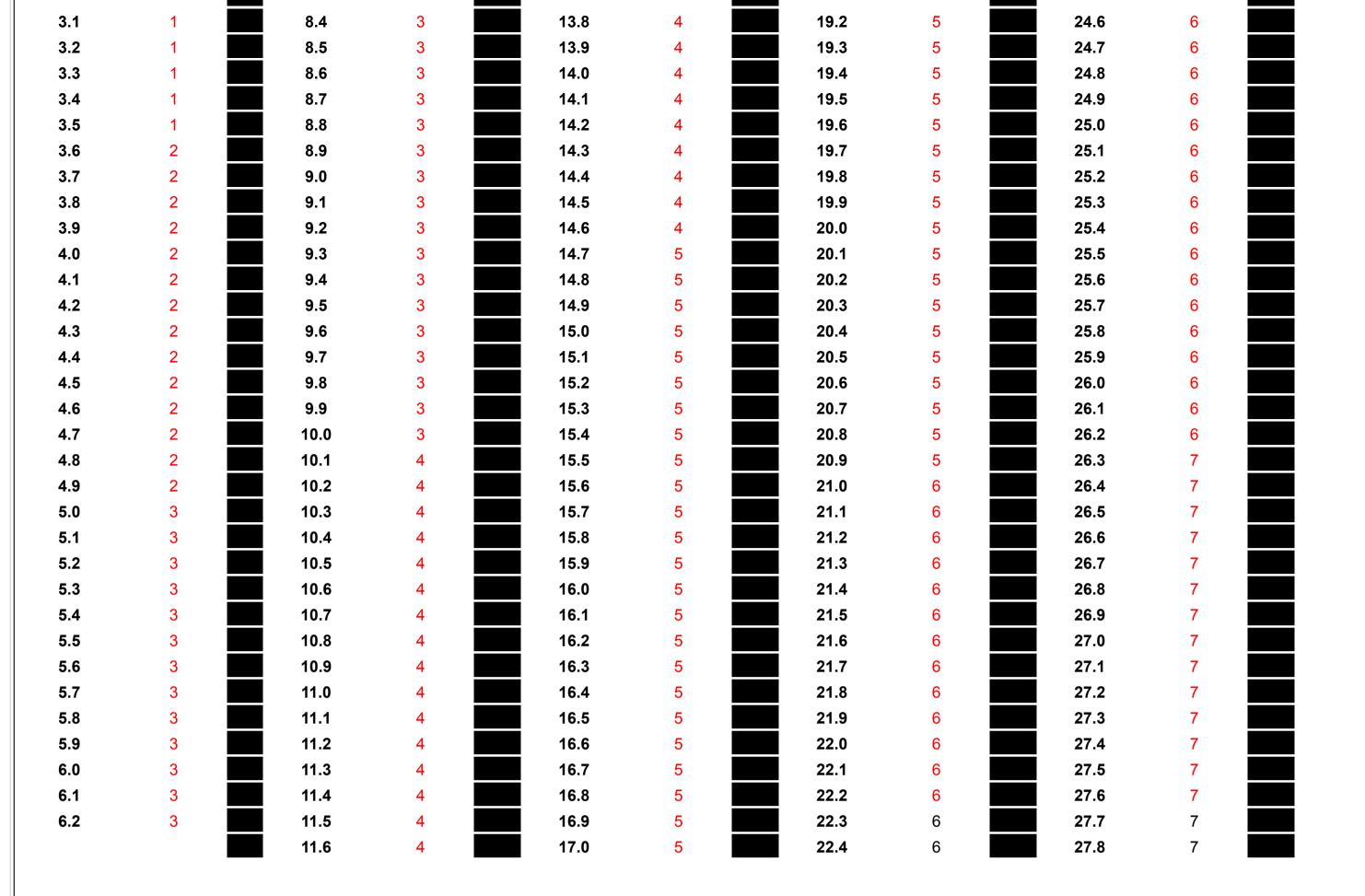
Relative progesterone concentrations during the proestrual/estrual period. P4 (ng/ml)

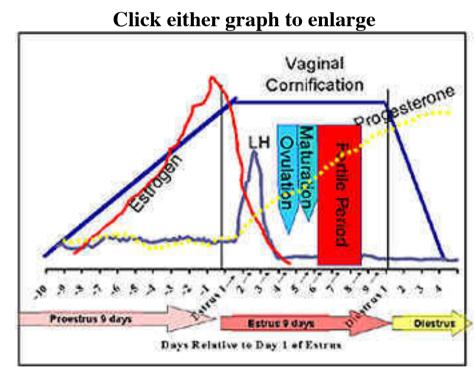
Relative time points
1.1-1.9 Before LH surge
2.1-2.9 Day of LH surge
3.1-3.9 Days 1-2 after the LH surge

4.0-8.0 Day of ovulation 10-80 2-3 weeks post-ovulation

Progesterone concentrations and day of cycle relative to LH peak (from

P4 ng	Day	P4 ng	Day	P4 ng	Day	P4 r	ig Day	P4 ng	Day	P4 ng
1.0	Pre-LH	6.3	3	11.7	4	17.		22.5	6	27.9
1.1	Pre-LH	6.4	3	11.8	4	17.2	2 5	22.6	6	28.0
1.2	Pre-LH	6.5	3	11.9	4	17.3	5	22.7	6	28.1
1.3	Pre-LH	6.6	3	12.0	4	17.4	1 5	22.8	6	28.2
1.4	Pre-LH	6.7	3	12.1	4	17.	5 5	22.9	6	28.3
1.5	Pre-LH	6.8	3	12.2	4	17.0	5	23.0	6	28.4
1.6	Pre-LH	6.9	3	12.3	4	17.7	7 5	23.1	6	28.5
1.7	Pre-LH	7.0	3	12.4	4	17.8	5	23.2	6	28.6
1.8	Pre-LH	7.1	3	12.5	4	17.9	5	23.3	6	28.7
1.9	Pre-LH	7.2	3	12.6	4	18.0	5	23.4	6	28.8
2.0	0	7.3	3	12.7	4	18.4	5	23.5	6	28.9
2.1	0	7.4	3	12.8	4	18.2	2 5	23.6	6	29.0
2.2	0	7.5	3	12.9	4	18.3	5	23.7	6	29.1
2.3	0	7.6	3	13.0	4	18.4	5	23.8	6	29.2
2.4	0	7.7	3	13.1	4	18.	5 5	23.9	6	29.3
2.5	0	7.8	3	13.2	4	18.0	5	24.0	6	
2.6	1	7.9	3	13.3	4	18.7	5	24.1	6	
2.7	1	8.0	3	13.4	4	18.8	5	24.2	6	
2.8	1	8.1	3	13.5	4	18.9	5	24.3	6	
2.9	1	8.2	3	13.6	4	19.0	5	24.4	6	
3.0	1	8.3	3	13.7	4	19.	5	24.5	6	





Progesterone testing (see Appendix C) **Qualitative**

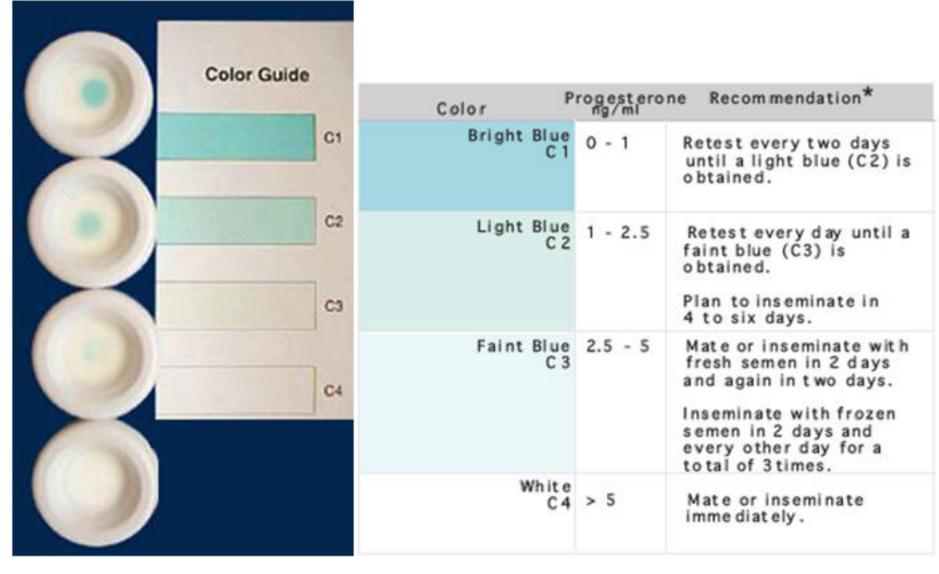
Hormone Analysis

- The initial progesterone rise during estrus in the bitch coincides with the LH peak.
- Before the LH peak the progesterone is < 1.0 ng/ml.
- On the day of the LH peak, progesterone rises to around 1.5 2.0 ng/ml. Thereafter the progesterone is > 5 ng/ml.
- Using our lab ovulation occurs at about 3.8-5.0 ng/ml (11.7 nmol/L) of progesterone (including the day of ovulation and the 24 hours it takes to ovulate) 24.1 ng/ml (54-74 nmol/L) of progesterone.
- The progesterone can be assayed quantitatively by a laboratory or qualitatively using an ELISA kit.
 - The ELISA kits are quick and semi-quantitative.
 - Use the ELISA kits as an adjunct to vaginal cytology.
 - Begin when vaginal cornification reaches 60 75%.

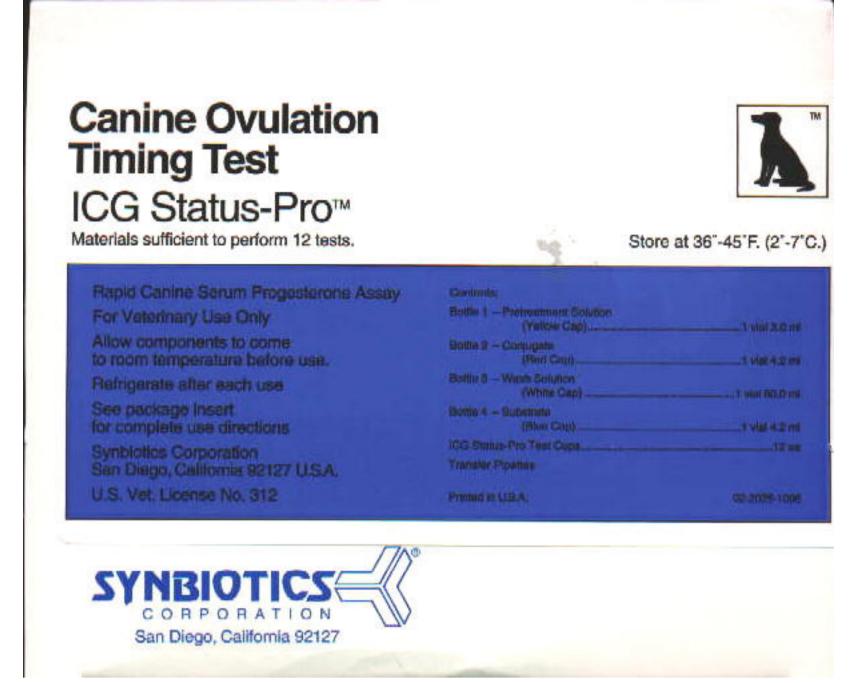
- If used alone, begin third or fourth day of proestrus. Test every other day.
- The 'Status-Pro' will indicate the day of if initial progesterone rise by the number of blue dots fading.
- The 'Target' test is read by the intensity of the dot color.

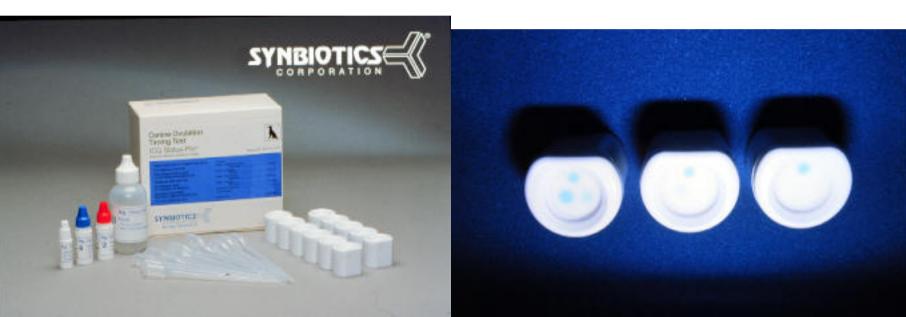
Target test





Status Pro

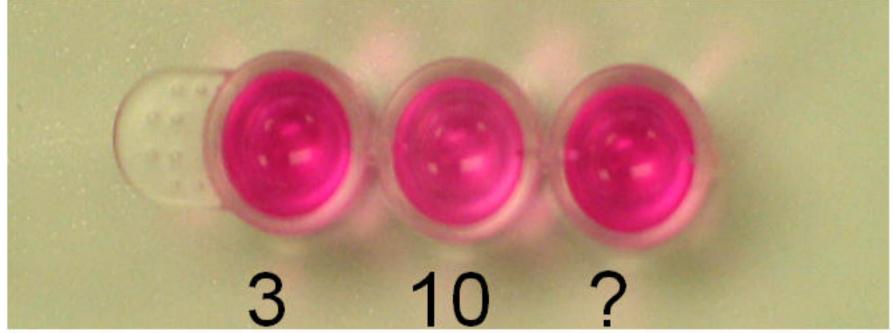




• The day the test goes from 3 blue dots to 2 blue dots is the day the progesterone went from < 1 ng/ml to 2-7 ng/ml, which coincides with the I

Premate





- 'Ovucheck Premate' has you read wells and compare the sample to a set of controls for color intensity.
 - It has ranges of < 3, 3-10 and > 10.
 - These ranges do not fit well with breeding protocols we use.
 - Handy to determine early vs late estrus.

K9 Proges-Check



- It is important to do follow-up testing to ensure that progesterone continues to rise, as sometimes the dogs become 'stuck' at 2-7 ng/ml. This may indicate
- It is also important to continue to follow the vaginal cytology.
 - Counting back 6 days from the first day of cytologic diestrus should coincide with ovulation. (This would be 8 days from the LH peak).
 - If these two tests do not predict ovulation on a similar day, then fertility may be compromised.
 - o If you skipped a day between tests and the test today is high and the test 2 days ago was low, then the day in between was the day of initial progestors.

USING PROGESTERONE KITS TO TIME INSEMINATION IN THE BITC



Dale Paccamonti, D.V.M., M.S., Dip. A.C.T.

When the number of breedings is reduced to one or two during a single estrus, such as with frozen or fresh cooled semen, timing insemination to coincide with ovulation becomes the LH surge. The bitch ovulates primary oocytes which require two to three more days for maturation. Mature oocytes are then viable for another two to three days. Therefore, the with peak fertility occurring five to six days after the LH surge.

Vaginal cytology does not give a very precise prediction of ovulation. The LH peak may occur anywhere from the same day, or up to two days after, full cornification. Unlike most bitch. Therefore, progesterone is useful to predict the LH surge as an increase in serum progesterone is closely associated with the LH peak. Before the LH surge, progesterone is rises to 1.5 to 2.0 ng/ml and thereafter continues to rise during diestrus or pregnancy. By identifying this initial rise in progesterone, the day of the LH surge can be estimated and fertility.

At least two kits are available for in-house, semi-quantitative progesterone testing. Both are CITE kits and are interpreted on the basis of color changes. The Target Kit (Biometal between different concentrations of progesterone on the basis of the color intensity of a single dot. A bright blue dot corresponds to a progesterone concentration of 0.0 to 1.0 ng/n ng/ml, and white: greater than 5.0 ng/ml. The Target kit includes an internal calibration which eliminates the need to run a control or to have multiple spots in the test. The Status 800-248-8099) uses three dots to differentiate between 0.0 to 1.5 ng/ml (3 dots), 2.0 to 7.0 ng/ml (2 dots) and greater than 7.5 ng/ml (1 dot) serum progesterone. The dot which is control.

Progesterone testing can be used as an adjunct to vaginal cytology or can be used alone if a practitioner is not comfortable interpreting vaginal cytology. If progesterone testing is should begin when vaginal cytology is approximately 60-75% cornified to establish a baseline with which to compare subsequent test results. If using progesterone tests alone, test procestrus.

Using the Status Pro kit, the low spot will begin to fade on Day 0 (the day of the LH peak) and will disappear on Day 1 post LH peak. The serum progesterone on Day 1 may occording the cases, though, rising progesterone can be detected by fading of the low progesterone dot. Using the Target kit, fading of the dot or the presence of a light blue dot corresport the LH peak.

Both tests come with concise, step-by-step instructions. The tests are easy to perform and results are available in about 20 minutes. Some aspects of the tests require attention to do come to room temperature before use. If the test is run using a cold kit, results will be incorrect, often giving a false high progesterone. The manufacturers recommend the kits be before use. Blood should be collected into a plain (red top) tube without anticoagulant for the Status Pro. For the Target kit, blood should be collected in either a plain (red top), E should be allowed to clot at a cool temperature (in the refrigerator) and cells should be separated from serum or plasma as soon as possible (within 20 minutes of collection is the remain with the red blood cells, progesterone will be bound by them and test results will be artificially low. With the Target kits, hemolyzed or lipemic samples may be used but a adding the enzyme (Step 3). Hemolyzed or lipemic samples will give a false low progesterone if used with the Status-Pro kits. Serum samples may also be frozen for analysis at a

If insemination is to be performed with fresh semen and multiple breedings are possible, insemination may be performed every other day after the initial rise in progesterone is obtains should be performed on either Days 3 and 5 or Days 4 and 6. Likewise, if insemination involves fresh chilled semen, fertility is best if breedings occur on Days 3 and chilled semen is reduced and timing of insemination is more critical. The viability of frozen semen is reduced even further and timing is even more critical. If multiple vaginal insemination, they should be performed on Days 4, 5 and 6. More commonly, a single surgical insemination is conducted when frozen semen is used. Surgical insemination with fro

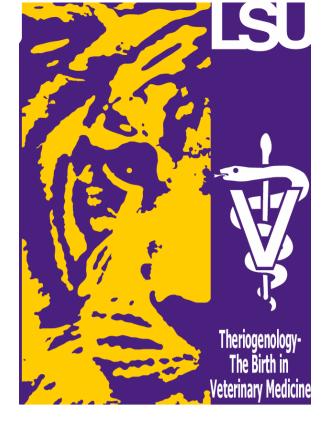
After the day of the LH surge is determined and insemination has been performed, vaginal cytology samples should be collected every other day until Day 1 of diestrus is determine retrospective, to determine the day of ovulation. Determining the day of ovulation by two methods and determining if they coincide may help in an infertility workup. Semi-quant weeks after the end of estrus to verify that progesterone is high when luteal insufficiency or ovulation failure are suspected.

Quantitative Progesterone

Some laboratories offer a 24 hour turn around on quantitative serum progesterone.

- Texas Diagnostic Lab
- Endocrine Diagnostics in Baton Rouge Veterinary Specialty in Mandeville
- Antec
- Local Hospital?

LSU SVM Theriogenology





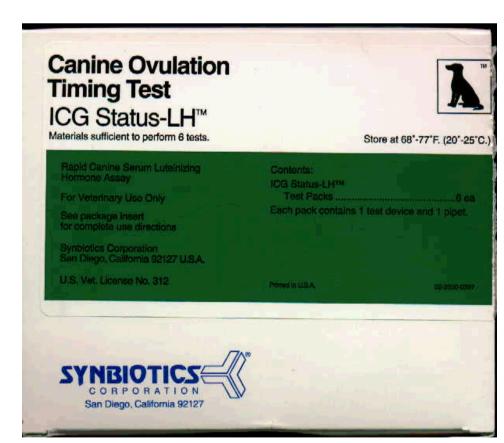
- Same day if notified by 12:00 and sample here by 3
- Saturday testing available if notified on Friday
- **◦** Current cost ~ \$40, \$70 for Stat samples (Saturday)

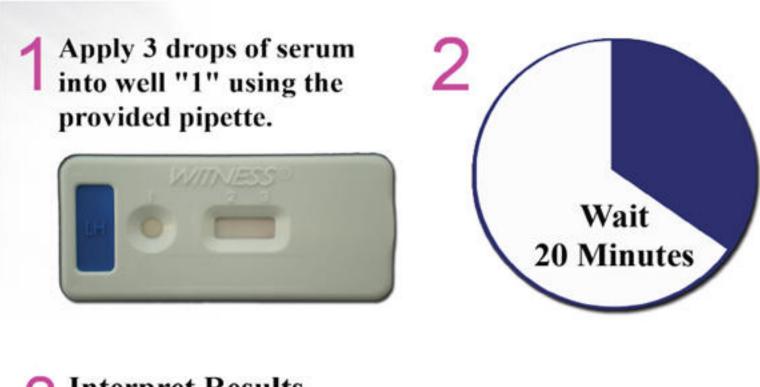
1.

Dr. Robert Hutchinson also states that the LH surges occurs when the quantitative progesterone is 2-3 ng/ml. However, Dr. Pat Concannon believes that it is the days that indicates the LH surge, not the value or magnitude. For example, it may increase from 0 .4 to 0 .8 ng/ml, 0.7 to 1.2 ng/ml, or 0.8 to 2.0 ng/ml. Other quantitative progesterone reaches 5.0 ng/ml. We have not been able to correlate quantitative progesterone that we have run to known ovulation dates, whereas

LH testing - Status LH or Witness



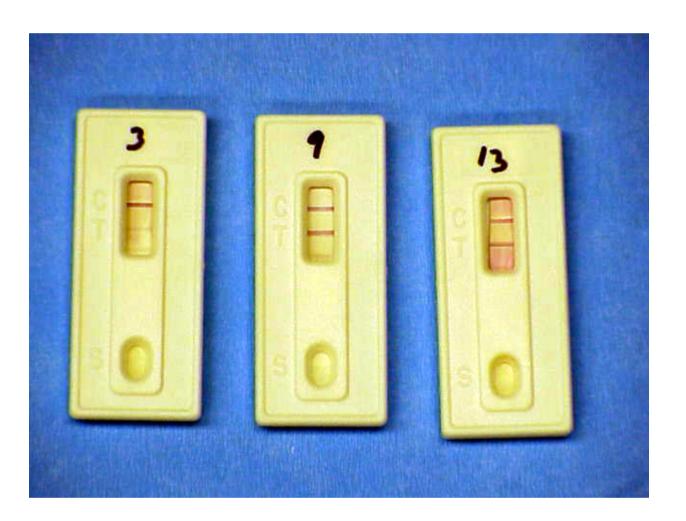






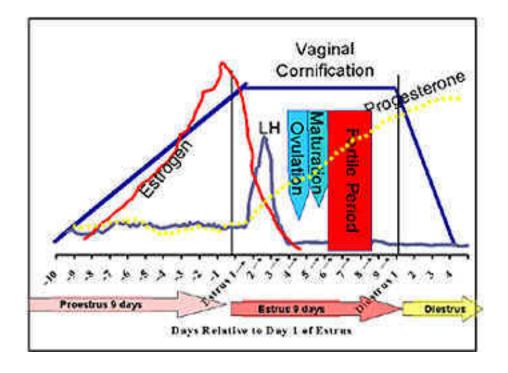






The Witness LH test is also a quick qualitative ELISA test. It must be run daily, because the LH has only a one day rise, and if a day of LH testing is missed reliable, but since testing must be done daily, it is usually more time consuming and expensive. If a single day is missed, the LH peak may have been missed determining the LH peak for us.

Click to enlarge

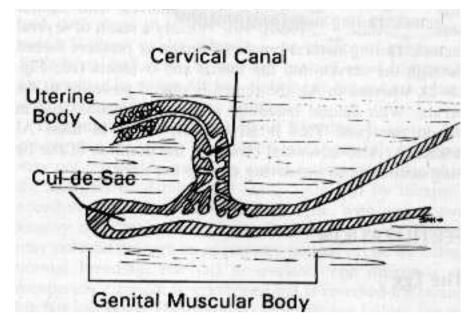


Breeding

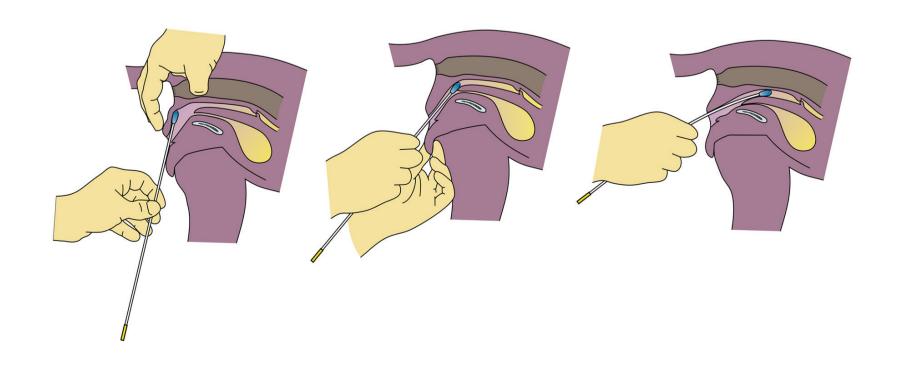
Artificial Insemination of Fresh Semen



- It is always best to use proven male, but always evaluate the semen to determine the number of cells and the quality of the cells inseminated.
- Breed every other day during estrus until the first day of cytologic diestrus.
 - Insert a pipette over the pubis and into the anterior vagina. Be careful to avoid urethra and inseminate the bladder, as pregnancy rates are low with
 - A cow AI sheath or a cut-off cow infusion pipette works well for most bitches.
 - Some smaller dogs may require a smaller pipette.
 - Commercial pipettes are available specifically for dogs.
 - Generally, an unskilled person working with regular equipment cannot enter the bitch's cervix. The cervix is difficult to enter because it is actually axis.



- Infuse the unextended semen.
- A regular syringe can be used because the sperm cells are not in contact with the syringe very long.
- Some syringes have been implicate as being spermacidal.
- It has been traditional to feather the dorsal vagina and to elevate the hindquarters for 10 minutes, but our research has shown neither of these to be
- As always, remember to do your AKC paperwork.
- How many cells are needed?
 - Vaginal AI on 3 consecutive days after acceptance of the male by the female with 50 X 10⁶ cells of fresh semen extended 1:4 resulted in lower ferti x 10⁶ cells of fresh semen extended 1:1 (80%) or natural mating (80 %).
- The volume fresh semen placed in the anterior vagina has not been critically evaluated.
 - Volumes as low as 2.2 ml and up to 3.6-3.9 ml have been reported to yield good pregnancy rates.
 - As long as an adequate number of cells is placed into the vagina, the volume of the inseminate does not affect fertility.
 - Excessively large semen volumes inseminated into the vagina could result in the drainage of some of the ejaculate from the vagina, however this has
 - There is only one controlled study that directly compared pregnancy rates of bitches bred by AI using fresh semen vs. natural mating, and it shower bitches mated by AI or natural mating when the same males were used under similar breeding conditions.
- Transcervical insemination (see below under Frozen Semen for more information)
 - Fertility after intrauterine insemination of fresh semen was greater than that obtained by vaginal AI with fresh semen, but the vaginal AI conception
 - Intrauterine insemination with fresh semen appears to have no benefits under most normal situations
 - One report indicates intrauterine insemination with fresh semen significantly improved the pregnancy rates in bitches that were previously infertile breeding other bitches.
 - At LSU (EVSSAR 2005 Budapest)
 - A total of 11 females during 82 estrous cycles (59 AI and 23 TCI) were evaluated.
 - Eleven different males were used for 1 to 22 estrous cycles (mean = 7, SD = 6.2). There was no difference among female or male fertility.
 - The median number of TCIs performed for each female (\pm SD) in the TCI group was 2 \pm 0.99.
 - The pregnancy rates for bitches in the AI and TCI groups were 38.3% (33/59) and 83.3% (20/23), respectively. The odds ratio of a female bed 0.005).
 - The average number of total breedings (\pm SD) in the TCI and AI groups was not different (4.3 \pm 1.1 and 3.9 \pm 1.4, respectively).
 - The average number of total cells inseminated (± SD) in the TCI and AI groups was not different (1802 ± 1085 and 1226 ± 1121, respectively





Click to see a video of artificial insemination in the bitch.

Breeding when only 1 or 2 breedings are available

(Limited availability of stud, shipped or frozen semen)

- If fresh cooled shipped semen is being sent in, it is important to predict the fertile period.
- The "Fertile period" is 4 to 8 days after the LH surge.
 - This is because the ovulation occurs 2 days after the LH surge and takes around 24 hours
 - Then the oocyte must undergo reduction division, which takes another 24 hours
 -hence at least 4 days past the LH surge.
 - The oocytes are viable for only a short though after the reduction division, so the peak fertility is 5 to 6 days after the LH peak.
- It is recommended to breed on days 3 and 5 or days 4 and 6 after the LH peak.
- The insemination procedure is the same as with fresh semen. There is no need to warm the semen before insemination, but a sample should be analyzed
- Results after using chilled extended semen can be the same as natural breeding if the proper number of cells are inseminated enough times.
- Swedish reports that summarized data from chilled semen inseminations show pregnancy rates can vary from 28-60% depending upon the type of extended
 - These pregnancy rates are lower when ovulation is timed and the number of breeding is limited than when compared to the 90-100% conception rates cells/breeding were used by us.





- After semen collection, analyze the sample and extend at least 1:1 in warm extender.
- We have found that the skim milk equine extender works as well as most of the commercial or home-made canine extenders.
- Package in a cooling system. Again, we have found the equine packaging systems work as well as the more expensive commercial canine packaging systems.
- At this time the AKC requires all studs that have semen chilled and shipped to be DNA tested before registration of pups is allowed.





Click on the AKC logo to go to the AKC Homepage

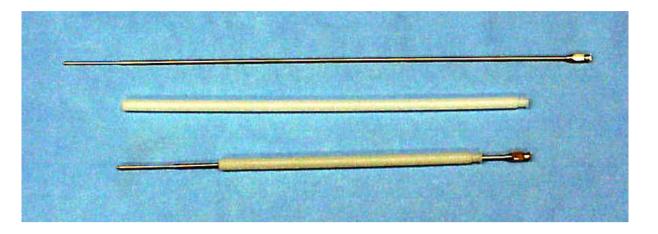




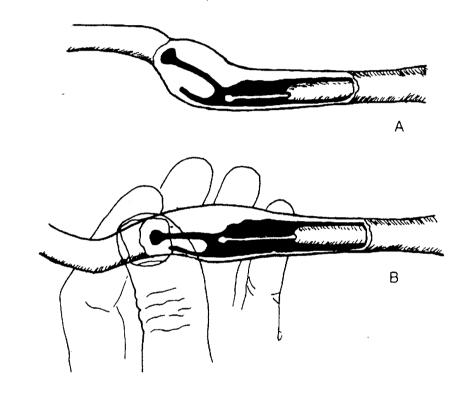


- If frozen semen is being used, surgical insemination generally gives the highest fertility.
- Since the frozen cells do not live as long, breed on day 5 or 6 after the LH peak.
- A ventral midline laparotomy is performed, the uterine horns exposed with minimal trauma, and the thawed semen is injected into either horn.
- Thaw the semen only when you are ready to inseminate and use the thawing directions supplied by the freezing company, no matter how you normally d
- Check the motility of a drop before insemination.
- Use only semen from AKC approved centers, or the puppies will not be able to be registered with the AKC.
- AKC paperwork is essential.
- Vaginal insemination with frozen semen generally has poor conception, because most people cannot penetrate the cervix.
 - With special training, however, some people have been able to obtain acceptable pregnancy rates if breedings occurred on days 4, 5, and 6 after the
 - A 1996 report in Veterinary Record by Silva, Onclin, Lejune and Verstegen state a 60 % conception rate using vaginal AI of 1 billion cells with 609
 - This was the same pregnancy rate using 400 million cells surgically inseminated.
 - A group of controls using fresh semen had 100 % conception rates.
- Norwegian Catheter
 - The catheter consists of a large plastic sheath and a smaller stainless steel catheter that fits inside the sheath.
 - There are at least three sizes of catheters made for different size dogs.
 - To perform the insemination
 - The sheath, with the internal catheter in place, is passed as far into the vagina as possible.
 - The tip of the stainless steel catheter is then advanced cranially into the fornix under the cervix.
 - Since the cervical os opens in a dorso-ventral direction, the catheter cannot be directly advanced through the cervix.

- The cervix must be palpated through the abdomen and grasped by the veterinarian.
- Once the cervix is grasped and the catheter is in the cervical fornix, the cervix is manipulated by turning in ventrally so the cervical os assume
- As the cervix assumes a horizontal orientation, the catheter is backed out of the fornix and threaded through the cervix
- When the catheter encounters the cervix, a 'gritty' sensation is felt by the veterinarian.
- Once the catheter is placed through the cervix, the semen is inseminated.
- The purchase of the catheters is a relatively small expense; however attaining the skill to consistently pass the catheter through the cervix requires
- The possibility of a vaginal or uterine rupture is always present when inexperienced clinicians are attempting this intrauterine insemination procedu
- Conception Rates
 - Intrauterine deposition of frozen semen yielded pregnancy rates of 67% breeding 1-2 times (the number of cells was not stated),
 - 83% breeding two times using 200 x 10⁶ cells/breeding
 - 84% breeding 1-3 times using 186 x 10⁶ cells/breeding.
 - Increasing the number of breedings from 1 to 3 did not significantly increase the conception rate using the Norwegian transcervical technique



The 'Norwegian' Catheter above

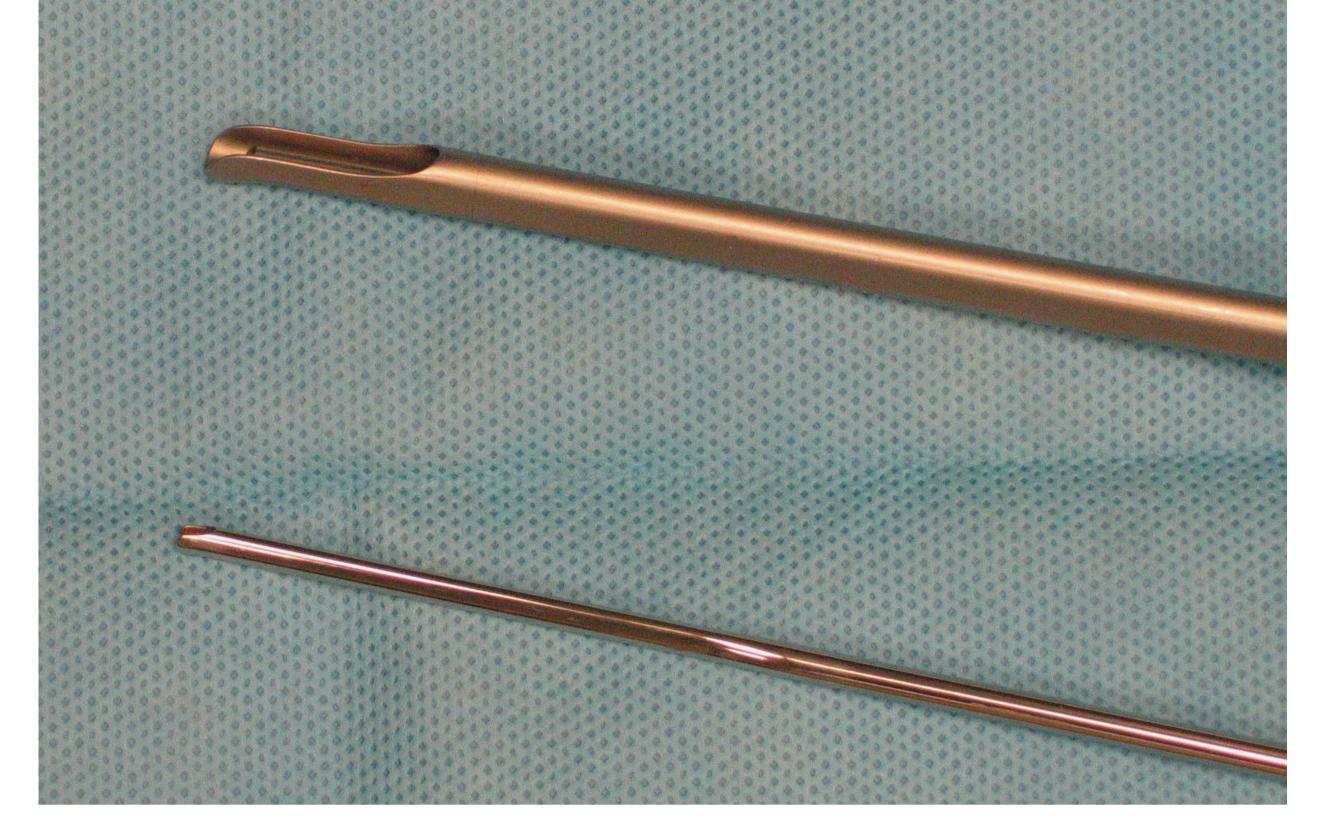


• A transcervical AI technique has been described by Marion Wilson from New Zealand.

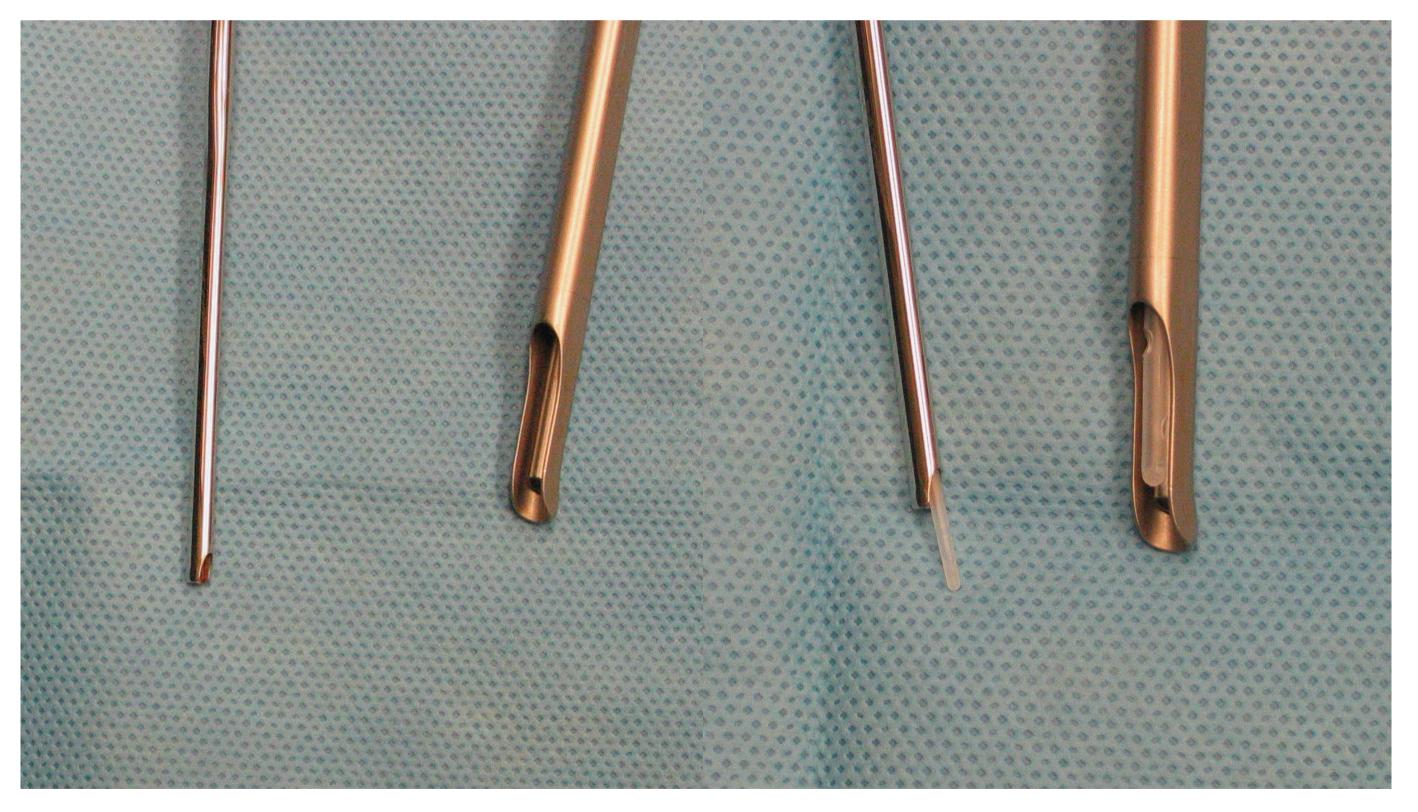


Click here to see a video by Marion Wilson of a TCI (edited by Eilts)

- \circ A 36 cm Storz cystoscope with a 30 $^{\circ}$ vewing angle is used.
- \circ A 55 cm scope with a 6^0 viewing angle is also used (smaller diameter) if the vagina is smaller, or more length is needed.



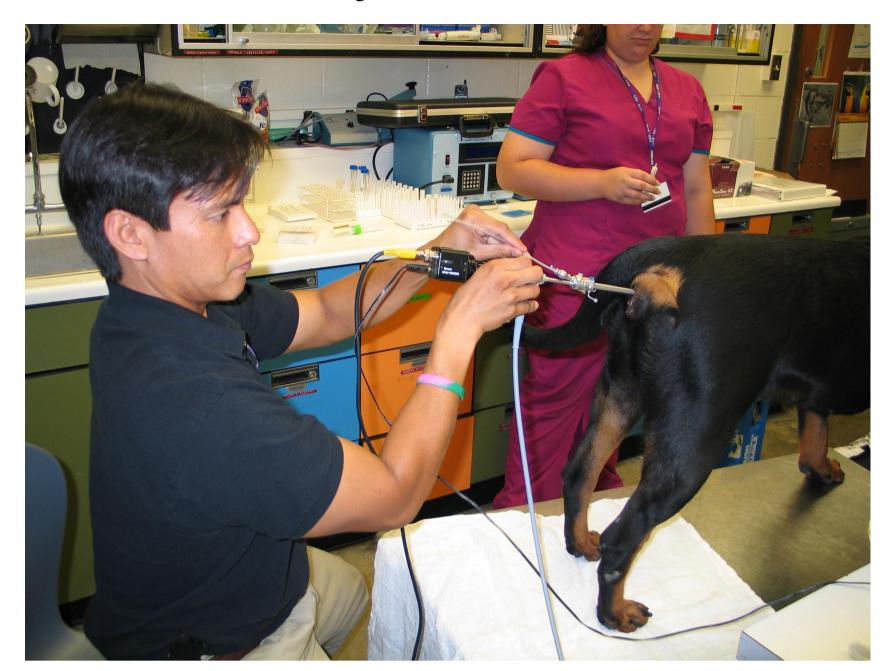
Smaller diameter 55 cm scope on bottom. Note the smaller diameter. The lens is at the end and the catheter channel enclosed just above the lens.

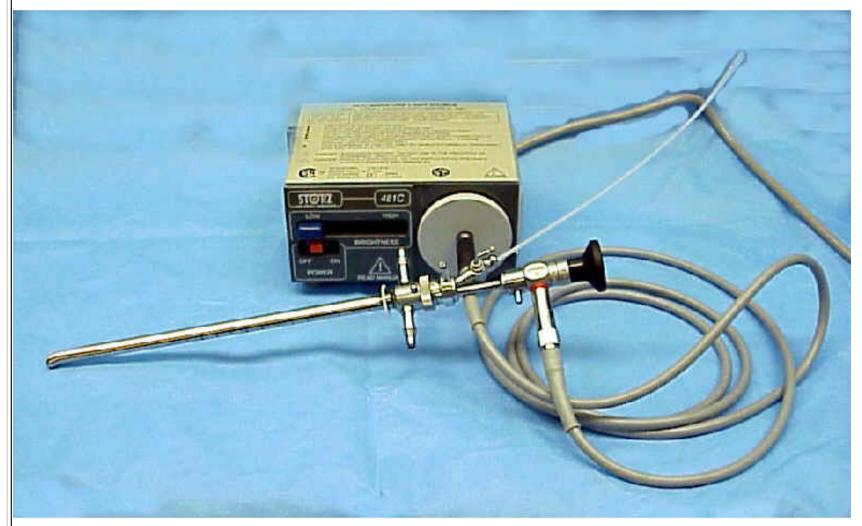


Smaller diameter 55 cm scope on left in each picture. The lens is at the tip and has a 60 viewing angle. The lens at the tip makes viewing slightly more d collapsing of the vaginal wall if the catheter is not used to hold the wall away. A smaller, longer catheter must be used.

- The cystoscope is passed into the vagina and the dorsal postcervical fold is identified.
- The cervical os appears as a rosette under the dorsal post cervical fold.
- An 8 fr catheter is used to inseminate
- Pregnancy rates
 - 100% breeding two times using as 200 x 10⁶ cells/breeding

- 85% using as few as $30-50 \times 10^6$ cells/breeding
- 57% (the number of breedings and dose was not stated)







36 cm cystoscope above. The lens is inset from the end of the sheath and the catheter channel is an open channel in the sheath.

- The standard dose for surgical AI is 100-200 million motile cells in one breeding 5-6 days after the LH peak. Most people use 100 million cells and bree progesterone is 5 ng/ml (15.9 nmoles/ml)
- LSU likes to breed with at least 100 million motile cells on days 5 and 6 post LH.
- Work from South Africa has shown that fertility can be obtained with as few as 50 million cells deposited vaginally, every day of estrus.
- The future of frozen semen probably is in multiple transcervical inseminations of lower doses of semen. This will also change the way we freeze cells (i.e.
- At this time all studs that have semen frozen, need to be DNA tested before registration is allowed.
- To register a litter with the AKC only semen from AKC approved semen centers can be used. There are a limited number of AKC approved centers, with

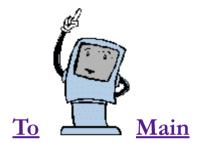
- The AKC only has bookkeeping requirements, they do not endorse any centers or have any quality control regulations.
- To become approved you must only show the AKC that you can keep accurate.

Click here to visit a site of Questions and Answers on Frozen Semen in the dog.

contributed by Bruce E Eilts on 21 September 2009



contributed by Bruce E Eilts on 25 September 2012



mailto:beilts@lsu.edu

Send an email to ask a question that has not been adequately covered.